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In-vitro Anti-oxidant and Free Radical Scavenging Activity of Lycopene

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ABSTRACT

Lycopene, C40H56, red fat soluble pigment found in **certain** plants and microorganisms. It is being used for the treatment of various disorders like cardiovascular diseases, osteoporosis, bone health, male infertility, skin protection, age related macular degeneration prevention, Alzheimer's disease, amyotrophic lateral sclerosis, asthma caused by exercise, immune stimulation, viral disease and DNA damage.[1] The THF extract of lycopene was subjected for in-vitro antioxidant activity using four different methods such **as** 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), nitric oxide radical, hydrogen peroxide radical and reducing power assay method. The THF extract of lycopene exhibited DPPH and hydrogen peroxide radical scavenging activity with IC50 value of 54.008µg/ml and 47.662µg/ml respectively. The lycopene showed significant antioxidant activity against nitric oxide radical with IC50 value of 57.879 µg/ml and antioxidant activity against reducing power method with IC50 value of 45.609 µg/ml respectively. Based on the results it can be concluded that THF extract of lycopene have good antioxidant effects against several oxidants.

Key words: DPPH; Hydrogen peroxide radical; Lycopene; Nitric oxide radical; Reducing power.

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INTRODUCTION

The link between the free radicals and disease process has led to considerable research with the aim to discover the nontoxic drugs that can scavenge the free radicals and thereby halt the causation and progression of the disease. In the cell the reactive oxygen intermediates continuously leak from the active sites of the enzymes involved in oxidative processes by inadvertently interacting with oxygen or other compounds. Stimuli known to increase the production of reactive oxygen intermediates include irradiation, ageing, and inflammation, raised partial pressure of oxygen, cigarette and reperfusion injury. Carbohydrates, membrane lipids, proteins and nucleic acids are vulnerable to damage caused by reactive oxygen species and this damage is believed to contribute to the pathogenesis of many diseases. Antioxidants remove free radicals and thereby terminate the chain reactions that can damage cells. [2] Practically natural antioxidants are obtained through the ingestion of plant products such as fruits, vegetables, nuts, flours, vegetable oils, drinks and infusions, taken fresh or as processed food stuffs.

The consumption of fruits and vegetables found to be associated with a lowered incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts. These protective effects are considered, in large part, to be related to the various antioxidants contained in them[3]. Lycopene, a red carotenoid pigment, C40H56 found in blood, the reproductive organs, tomatoes and palm oils [4]. It is a Carotenoid without provitamin A activity and present in many fruits and vegetables. It is a red fat soluble pigment found in certain plants and microorganisms, where it serves as an accessory light gathering pigment and protect the organisms against the toxic effect of oxygen and light [5]. As an antioxidant its consumption can reduce the risk of some cancers. The FDA has approved Generally Recognized as Safe (GRAS) status to lycopene. Recently the FDA has also given a limited health claim declaration for lycopene, stating “very limited and preliminary scientific research suggests that eating one of the cup of tomatoes and/or tomato sauce a week may reduce the risk of prostateCancer” [6]. Consuming cooked tomato sauces, tomato ketchup, tomato soup, stewed tomatoes and other cooked tomato dishes are excellent sources of lycopene [7]. Lycopene at physiological concentrations can inhibit human cancer cell growth by interfering with growth factor receptor signaling and cell cycle progression specifically in prostate cancer cells without evidence of toxic effects or apoptosis of cells. A gene, connexin 43 in human body is up regulated by lycopene and which allows direct intercellular gap junctional communication (GJC). GJC is deficient in many human tumors and its restoration or up regulation is associated with decreased proliferation [8]. The THF extract of lycopene was evaluated for its free radical scavenging activities by DPPH, nitric oxide, reducing power method and hydroxyl scavenging assays. Our present study reports antioxidant potential of lycopene using different invitro methods. Ascorbic acid was used as a standard.

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MATERIALS AND METHODS

Materials

Lycopene: The drug lycopene (6%) CWD powder was purchased from the Bio-Gen Extracts Pvt. Ltd. (Bangalore)

Chemicals

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) was obtained from the Sigma Aldrich Co, St Louis, USA. Ascorbic acid was obtained from S.D. Fine Chem., Biosar, India. Hydrogen peroxide (30%) was obtained from Qualigen Fine Chemicals, Mumbai, India. Sodium nitroprusside (SNP) was purchased from Merck Ltd. India, Mumbai. Potassium ferri cyanide and trichloroacetic acid was obtained from S.D. fine-chem. Ltd. All chemicals and solvent were of analytical grade obtained from SRL and Merck, Mumbai, India.

IN-VITRO ANTIOXIDANT ASSAY

DPPH radical scavenging activity

The ability of the extract to scavenge DPPH radicals were determined by the method of Gyamfi et a [19] with the minor modifications. A 0.5ml aliquot of test extract at different concentrations in cyclohexane was mixed with 0.5 ml of 100 mM methanolic solution of DPPH. After 30 minutes incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517nm. The percentage inhibition was calculated using the following formula:

Percentage inhibition = [Abscontrol - Abssample] /Abscontrol x 100

Where, Abscontrol was the absorbance of solution without extract and
Abssample was the absorbance with different dilutions of drug extract.

Determination of nitric oxide radical scavenging activity

The compound SNP is known to decompose in aqueous solution at physiological pH (7.2 an) producing nitric oxide radicals (NO). Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate d nitrite). The quantities of which can be determined using Griess reagent. The scavenging effect of lycopene extract on nitric oxide was measured according to the modified method of Morocci et al. [10], 1 ml extract solution at different concentrations were added in the test tubes to 1 ml of SNP solution (100mM) and the tubes were incubated at 29°C for 2.5 hr. An aliquot of I ml of incubation solution was removed and diluted with I ml of Griess reagent (1% Sulfanilamide in 2% H_3PO4 and 0.1% N-1-Napthylethylenediamine dihydrochloride). The absorbance of chromophore that formed with

Napthylethylenediamine dihydrochloride was immediately read at 540 nm. The percentage inhibition was calculated using the formula:

Percentage inhibition = [Abscontrol - Abssample] / Abscontrol x 100

Where, Abscontrol was the absorbance of solution without extract and
Abssample was the absorbance with different dilutions of drug extract.

Scavenging of Hydrogen peroxide

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Various concentrations of 1 ml of the extract or standard in were added to 2 ml of hydrogen peroxide solution in PBS. Te absorbance was measured at 230nm after 10 minutes against a blank solution that contained extract or standard in PBS without hydrogen peroxide. IC50 values were calculated. [11]

Reducing power assay method

Different concentrations (10-50µg/ml) of lycopene extract were prepared and 1 ml of each solution was mixed with phosphate buffer (2.5ml, 0.2M, Ph 6.8) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. To this mixture, 2.5ml of 10% trichloroacetic acid (TCA) was added and then centrifuged at 3000rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5 ml, 0.1%) was added and the absorbance was measured at 700nm.

The percentage of reducing power was calculated by using the formula:

Reducing power (%) = Abscontrol - Abssample / Abscontrol [12]

Where, Abscontrol was the absorbance of solution without extract and
Abssample was the absorbance with different dilutions of drug extract.

RESULTS AND DISCUSSIONS

In-vitro antioxidant activity

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging assay is the most widely used method for screening antioxidant activity, since it can accommodate many samples in short period and detect active ingredients at low concentration [13,14]. The decrease in absorbance of DPPH radical scavenging caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a color change from purple to yellow. The THF extract of lycopene showed DPPH radical scavenging activity in a concentration dependent manner (fig. 2), with the correlation coefficient value (r) of 0.987 and IC50 value of 53.341 µg/ml. The IC50 value for ascorbic acid was 54.008 µg/ml.

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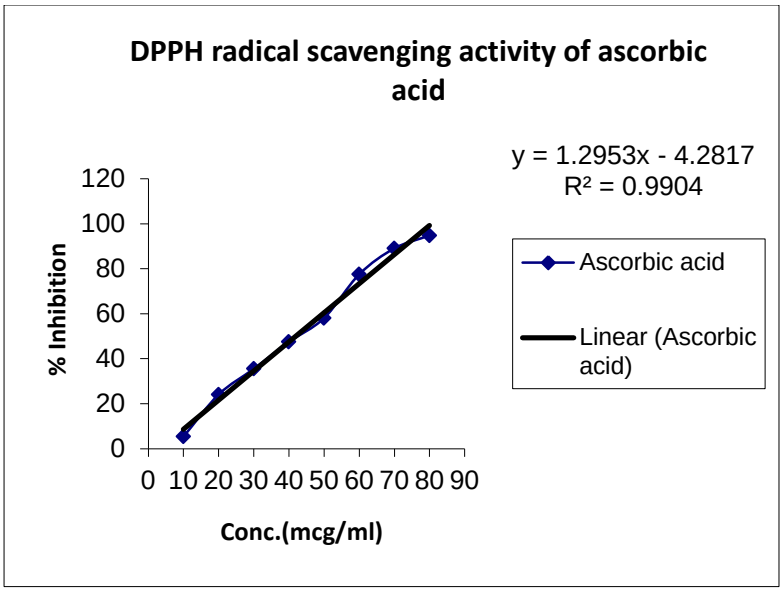

Figure 1 DPPH radical scavenging activity of ascorbic acid as a standard

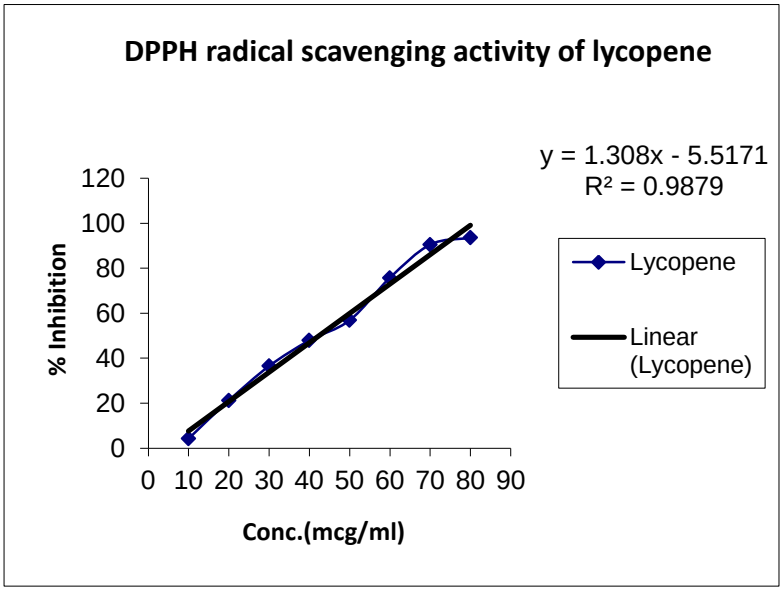

Figure 2 Effect of lycopene extract on 1, 1-Diphenyl-2-Picrylhydrazyl radical scavenging activity

Determination of nitric oxide radical scavenging activity

The effect of THF extract of lycopene on nitric oxide radical scavenging activity as shown in fig. 4. The compound SNP is known to decompose in aqueous solution at physiological pH (7.2) producing nitric oxide radicals (NO). Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite). This lead to reduction of nitrite concentration in the assay media [15]. Here, THF extract of lycopene exhibited nitric oxide radical scavenging

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activity with correlation coefficient values (r) of 0.972 and IC50 value of 57.879 µg/ml. The IC50 value for ascorbic acid of 53.568 µg/ml.

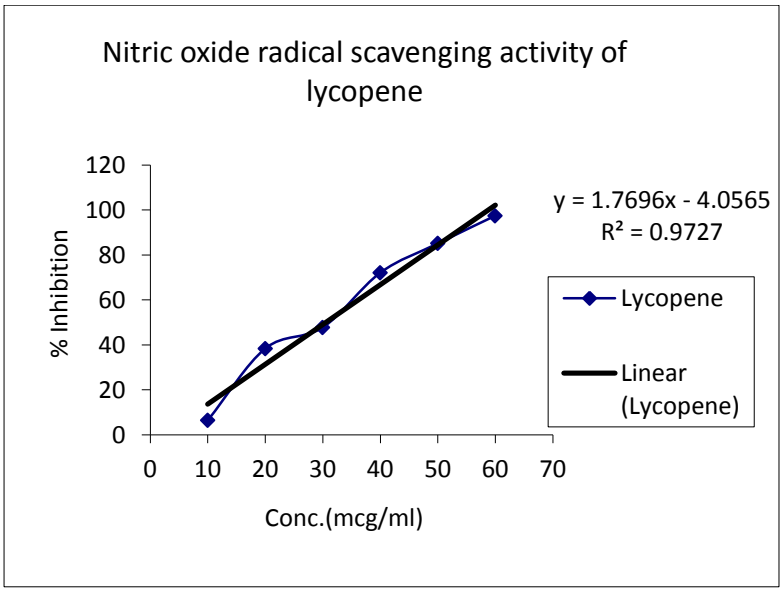

Figure 3.Nitric oxide radical scavenging activity of ascorbic acid as a standard

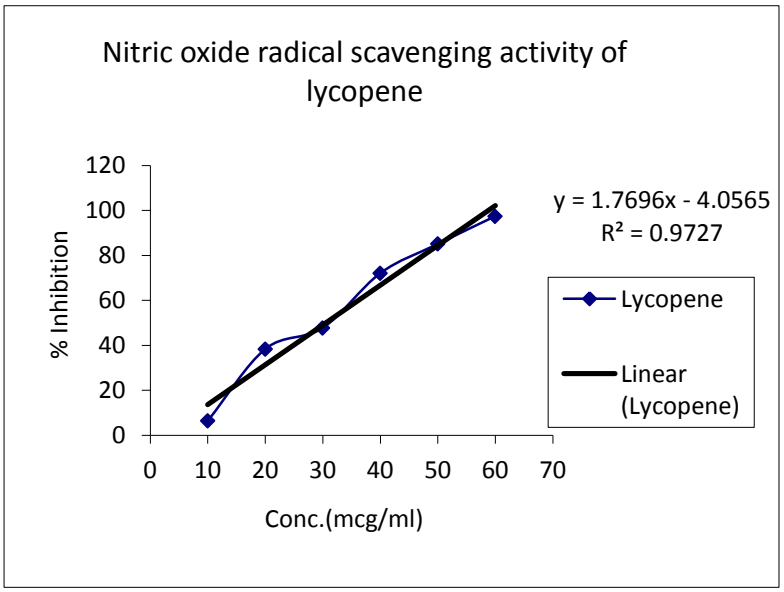

Figure 4.Nitric oxide radical scavenging activity of lycopene extract

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Scavenging of hydrogen peroxide

The effect of THF extract of lycopene on hydrogen peroxide radical scavenging activity as shown in fig. 6. The THF lycopene extract showed significant antioxidant activity against H2O2 radical with correlation coefficient values (r) of 0.986 and IC50 values of 36.554µg/ml, comparable to IC50 value of ascorbic acid of 47.662 µg/ml.

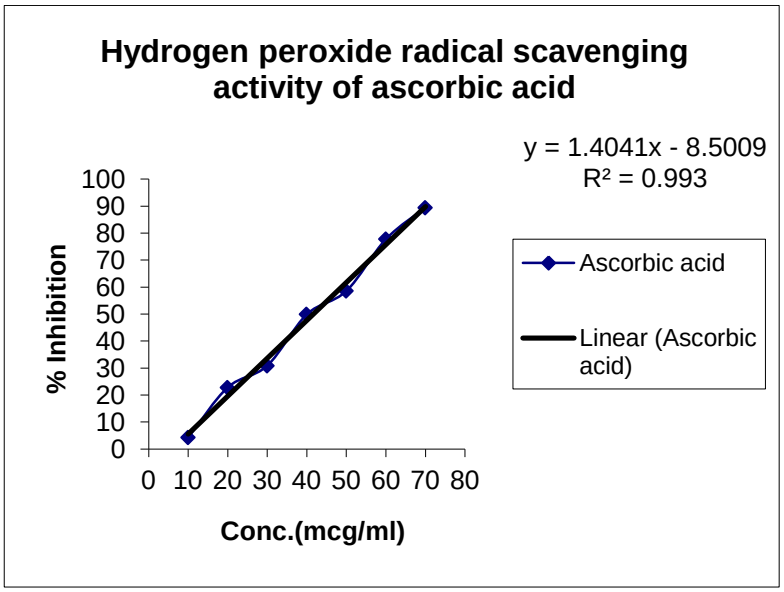

Figure 5. Hydrogen peroxide radical scavenging activity of ascorbic acid as a standard

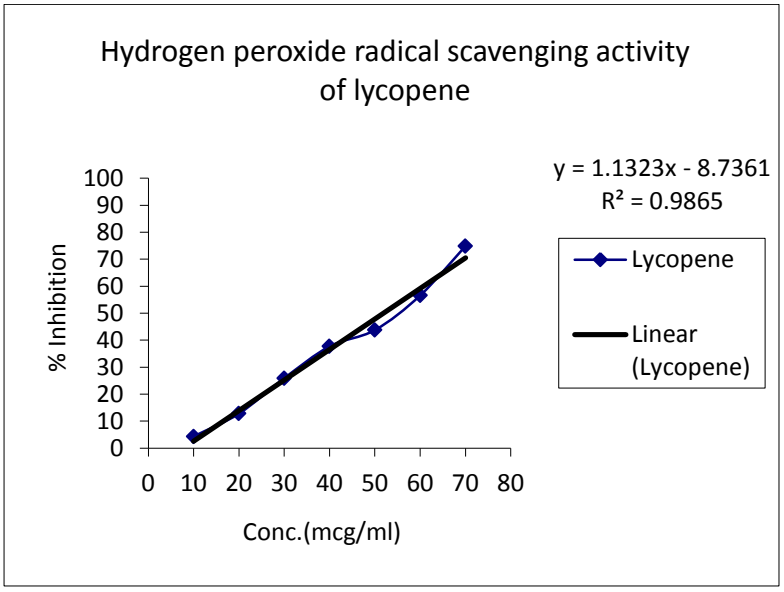

Figure 6. Hydrogen peroxide radical scavenging activity of lycopene extract

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Reducing power assay method

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. In the reducing power assay, presence of antioxidants in the samples would results in the reducing of Fe^{3-} to Fe^{2-} by donating an electron. Amount of Fe^{2-} complex can be then be monitored by measuring the formation of Prussian blue at 700nm indicates an increase in reductive ability [16]. Fig. 8 shows dose-response curve for the reducing power of the extract. It was found that the reducing power of all the extract also increased with the increase of their concentrations. All extracts had shown good reducing power that was comparable with ascorbic acid. The THF lycopene extract showed significant antioxidant activity with correlation coefficient values (r) of 0.985 and IC50 value of 45.921 µg/ml. The IC50 value of standard ascorbic acid showed IC50 value of 48.908 µg/ml.

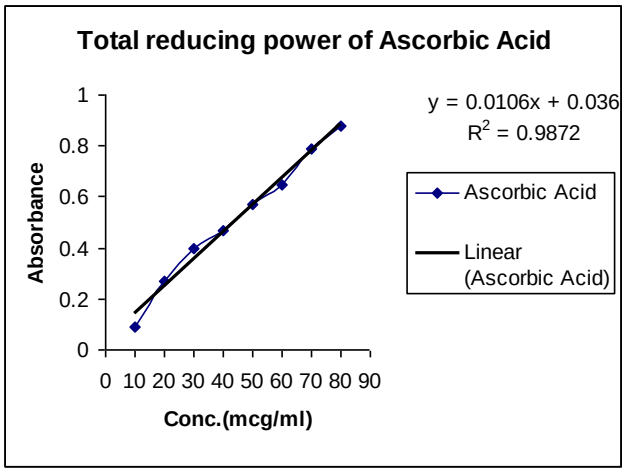

Figure 7. Total reducing power of ascorbic acid as a standard

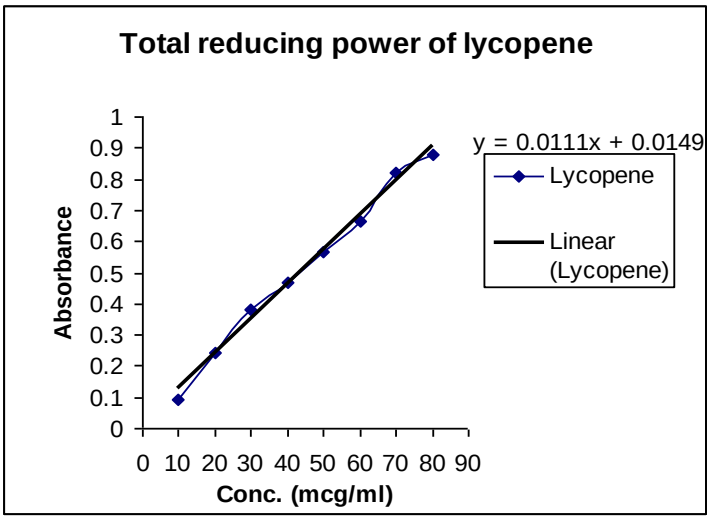

Figure 8. Total reducing power of lycopene extract

From these results, Lycopene found to possess comparable free radical scavenging activity as that of standard ascorbic acid in all the models.

REFERENCES

[1] Amarjeet Kaur, Jatinder Dhari, Om Prakash Sharma, GD Gupta, Vandana Kharb. IJPT 2011; 3 (4); 1605-1622.

[2] Edakkadath R Sandhu, Korengath C Preethi, Ramadasan Kuttan. International Journal of Experimental Biology 2010; 48: 843-848.

[3] M Ayub Ali, L Inaotombi Devi, Varij Nayan, KH Victoria Chanu, Laisanglura Ralte. International Journal of Biological and Pharmaceutical Research 2010; 1(2): 76-81

[4] The American Heritage @ Dictionary of English Language 4th Edition by Houghton Muffin Company updated in 2009, published by Houghton Miffin Company.

[5] En. Wikipedia.org/wiki/Lycopene

[6] Barbaro L Dillingham and A Venket Rao. INT JNM 4(1);2009

[7] www.livestrong.com/articles/362157. Good sources of lycopene

[8] Haber D, Lu Qy. Exp Biol Med (Maywood) 2002; 227 (10): 9203.

[9] Gyamfi MA, Yanamine M and Aniya Y. Genetic Pharmacy 1999; 32: 661-667

[10] Marcocci J, Maguire JJ, Droy- Lefaix MT and Packer L. Biochem Biophys Res Commun 1994; 201: 748-755

[11] Guddadarangavvanahally KJ, Lingamallu JR, Kunnumpurathu KS. Bioorg Med Chem 2004; 12: 5141-5147.

[12] KNV Roa, R Aradhana, David Banjii, RSNAKK Chaitanya, A Anil Kumar. JPR 2011; 4(2): 440-442

[13] Piao XL, Park IH, Baek SH, Kim HY, Park MK and Park JH. J Ethanopharmacol 2004; 93: 243-246

[14] Yu L, Haley S, Parret J, Harris M, Wilson J and Qian M. J Agri Food Chem 2002; 50: 1619-1624

[15] Sachin S Sakat, Archana R Juvekar and Manoj N Gambhire. Int J Pharm Pharm Sci 2010; 1(1): 146-155

[16] Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M and Eslami B. Phcog Mag 2009; 4(18): 123-127.